

Mechanism of Reduction of Cut Rose Flower Longevity as Affected by Brittle Leaf Formation

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Abstract

One of the major problems of winter-harvested rose flowers is occurrence of brittle leaves, which appear less waxy and occur more frequently in high humidity growing conditions. Commercial rose cultivars were tested to investigate the mechanism of the reduction of cut flower longevity caused by brittle leaf formation.

Water loss of detached brittle leaves from cut 'Asami Red' flowers increased during three hours after harvest, and significant water loss began to show even within one hour compared to that of normal leaves. This symptom was more severe in the leaves from lower nodes. There was no significant difference between brittle and normal leaves in leaf area or stomatal number in the same cultivar. The longevity of cut rose flowers with brittle leaves was improved by removing all the leaves below the second node. However, pretreatment with RNA-Ag+tris(hydroxymethyl)-aminomethane inhibited excessive water loss of brittle-leaved cut 'Asami Red' flowers, hence significantly improving flower longevity even when the brittle leaves were retained.

Large variation in brittle leaf formation was observed among cultivars, ranging from 0 to 40 %. Differences in flower longevity among cultivars were not related to brittle leaf formation. Further, the occurrence of brittle leaf was independent of leaf morphological characteristics.

INTRODUCTION

Longevity of cut rose flowers is often influenced by seasonal variations (Urban and Brun, 1995). Postharvest life declines from October through March in the Netherlands mostly due to leaf drying (Slootweg et al., 2001), also known as brittle leaf. High relative humidity (> 85%) and continuous lighting during the growing season are major causes of brittle leaf formation, inducing failure of stomatal closure in darkness and high transpiration rate (Mortensen and Fjeld, 1995; Mortensen and Gislerod, 1999). Continuous opening of stomata can be a problem after harvest since it causes severe water stress in cut flowers (Mayak et al., 1974; Slootweg and van Meeteren, 1991).

When transpiration rate is higher than that of water uptake, water balance between water uptake and water loss is disrupted (Van Doorn, 1997). However, it is unclear how water balance is affected by brittle leaf formation in cut rose flowers. Further, most research focuses on factors relating to growing conditions (Mortensen and Fjeld, 1995; Mortensen and Fjeld, 1998; Mortensen and Gislerod, 1999; Slootweg et al., 2001), and only a few suggest methods to reduce negative impact of brittle leaves when they occur (Torre and Fjeld, 2001). Our unpublished results showed that use of RNA-Ag+trishydroxymethyl-aminomethane (=tris) (Meiji Milk Co., Odawara, Japan) dramatically improved longevity of cut rose flowers by maintaining water balance. Since the main cause of the reduction in longevity of brittle-leaved flowers is the disruption of water balance, we hypothesized that pretreatment with RNA+Ag-tris would improve postharvest quality of brittle-leaved cut rose flowers as well.

'Asami Red' is one of the most popularly grown rose cultivars in Japan. However, flower longevity is often terminated by brittle leaf formation during winter. In this study, we investigated the mechanism of longevity reduction in cut 'Asami Red' rose flowers as affected by brittle leaf formation, focusing on water balance. RNA-Ag+tris was evaluated

to reduce negative effects of brittle leaf formation. Further, brittle leaf formation was examined with six commercial rose cultivars.

MATERIALS AND METHODS

Hydroponically grown rose (*Rosa hybrida* L.) flowers, cv. Asami Red, Black Tea, Ducut, Jeid, Tineke, and Pareo'90, from a rockwool culture system were obtained from commercial rose growers in Shimizu, Japan. Relative humidity was recorded with humidity sensor (RS-11, ESPEC) and was $80 \pm 1\%$ from November through January when experiments were conducted. The humidity increased above 85 % from the beginning of December for about 10 days.

Flowers were harvested at the tight-bud stage, when the buds show full color but the petals have not yet started unfolding, and transported to the laboratory immediately after harvest. Stems were trimmed to a length of 50 cm and leaves on the lowest 20 cm of the stem end were removed leaving 3 or 4 leaves on the stem. The removed leaves were used to identify normal or brittle leaf by measuring the change in water loss. The leaves were suspended in the air maintained at 20°C, 40-50 % RH with continuous lighting from cool white fluorescent lamps at $3-6 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the change in fresh weight was monitored for 3 hours. Relative water loss was calculated by taking a proportion of the change in fresh weight. The leaf was dried at 60°C and dry weight was measured after 48 hours.

After normal- and brittle-leaved flower stems were identified by the above procedure, they were pulsed with distilled water or 100 ppm RNA-Ag+tris (Meiji Milk Co., Odawara, Japan) for 8 hours. The flower stems were placed in a shipping box and subjected to simulated transport for 18 hours at 20°C, 40-50 % RH in darkness. Stems were recut to a length of 40 cm and placed in tap water at the same conditions as described above. Water uptake, fresh weight, and flower longevity were monitored daily for 7 days. Flower longevity was defined as the time from the first day after simulated transport to the day showing flower wilting, bud opening failure, blueing, blackening, or bent neck.

To measure water loss during the postharvest evaluation period, three or four leaves were taken from normal- or brittle-leaved flower stems and placed in test tubes filled with tap water. Fresh weight and water uptake were measured during the experimental period. At the end of experiment, the leaves were scanned with a scanner and leaf area was calculated by counting the number of pixels with Adobe Photoshop (Adobe Photoshop 5.5, Adobe Systems Inc.).

To investigate the difference in morphological characteristics of stomata between normal and brittle leaves, a replica was made from an attached leaf at the third or fourth node by applying clear nail varnish to both abaxial and adaxial parts of each pointed leaflet. The replica was carefully removed, and its picture was taken with a digital camera (Coolpix 950, Nikon) connected to the microscope and the image was analyzed on the computer. Stomatal size and number, and the extent of stomatal opening were measured.

Statistical analysis was performed with MINITAB (MINITAB® for windows, Minitab Release 13, Minitab Inc.) at $P < 0.05$.

RESULTS

Water loss of both normal and brittle leaves increased at the same rate during the first 30 minutes after harvest (Fig. 1). However, brittle leaf continuously intensified water loss during the next 3 hours while normal leaf subsequently ceased water loss. Our microscopic observation showed that stomata of normal leaves closed within 30 minutes, but that of brittle leaves failed to close throughout the experimental period (data not shown). There was no difference in morphological characteristics, such as stomatal number and size between normal and brittle leaves (data not shown).

Leaf area increased basipetally along the stem. There was no difference in leaf area between normal and brittle leaves (data not shown). Water loss of leaves in both groups increased toward the stem end, but brittle leaves from second to fourth node

showed significantly higher water loss compared to normal ones (Fig. 2). However, water loss per unit area remained the same in normal leaves regardless of the leaf position, while that of brittle leaves considerably increased in the lower leaves. Since water loss in brittle leaves from third to fourth node was particularly high, ranging from 40 to 60 % (Fig. 2), those brittle leaves were removed for further experiments to observe the effect on cut flower longevity. The longevity of brittle-leaved flower was significantly reduced compared to normal-leaved one (Fig. 3). However, the removal of leaves from third to fourth nodes increased the longevity of brittle-leaved flowers by 1.6 times. When the leaves at the third and fourth nodes were removed from normal-leaved flower stems, flower longevity slightly increased as well.

Pretreatment with RNA+Ag-tris significantly improved flower longevity in both normal- and brittle-leaved flowers (Table 1). The longevity was dramatically improved in brittle-leaved flowers even when the leaves were retained, and was even higher than that of normal-leaved ones not pretreated with RNA+Ag-tris. Water balance of cut flowers in all treatments dramatically decreased within the first day of the postharvest evaluation period (Fig. 4). Brittle-leaved flowers showed further decrease to below 0 by the second day along with a significant reduction of fresh weight (data not shown), while other treatments remained constant in water balance. RNA+Ag-tris maintained water balance in brittle-leaved flowers equal to normal-leaved flowers pretreated with or without RNA+Ag-tris (Fig. 4).

The occurrence of brittle leaves varied with cultivar, and was high in 'Ducut' (38.5 %) and 'Asami Red' (20.5 %), low in 'Tineke' (5.0 %) and 'Jeid' (4.8 %), and not observed in 'Black Tea' and 'Pareo'90' (Fig. 5). Water loss from detached leaves tend to increase in the cultivars with higher occurrence of brittle leaf ($r^2=0.6$), and cultivars with higher number of stomata seemed to be associated with higher chance of brittle leaf formation ($r^2=0.5$) (data not shown). However, the correlation was not significant at $P<0.05$ level. Flower longevity varied among the tested cultivars, and was not related to the extent of brittle leaf formation (Fig. 6).

DISCUSSION

Our results demonstrate that normal leaves can reduce water loss by closing stomata within 30 minutes after harvest, but brittle leaf does not reduce the rate of water loss after harvest due to failure of stomatal closure. Since brittle leaves develop at the lower nodes of the flower stem, prompt removal of the leaves below the second node can reduce water loss, improving flower longevity. However, the removal of lower leaves may not be appropriate in horticultural practice since it can reduce the value of cut rose flowers as a commercial product.

Pretreatment with RNA+Ag-tris significantly improved flower longevity in brittle-leaved flowers (Table 1). RNA+Ag-tris not only decreased water loss but also reduced water uptake in both normal- and brittle-leaved flowers in comparison with normal-leaved flowers without the same treatment, while brittle-leaved flowers showed less water uptake compared to the amount lost by transpiration (data not shown). Therefore, it is concluded that RNA+Ag-tris improves flower longevity by reducing both water uptake and water loss and stabilizes water balance. However, it is unclear whether RNA+Ag-tris reduced water loss by affecting stomatal behavior. Not only transpiration rate but also vascular occlusion and air embolism are determining factors of water uptake in cut rose flowers (Van Doorn, 1997). RNA+Ag-tris contains silver ion, a bactericidal agent (Veen, 1979). Since the mobility of silver ion in RNA+Ag-tris is lower than that of STS, it is more effective to reduce bacterial population at the stem end (Ohkawa et al., 1999). Therefore, significant improvement of flower longevity in both treatments might be partly due to a bactericidal effect of RNA+Ag-tris.

From the above results, it is concluded that RNA+Ag-tris would be an effective agent to improve postharvest quality of brittle-leaved cut rose flowers. However, since the effects of RNA+Ag-tris on the longevity of brittle-leaved flowers varied from one year to another and symptoms of toxicity was observed in some experiments, more thorough

examination should be done to solidify beneficial effects of RNA+Ag-tris.

The occurrence of brittle leaf varied among cultivars, ranging from 0 to 40% (Figure 4). However, the occurrence of brittle leaf was not correlated with stomatal number, stomatal size or water loss of detached leaves in the tested cultivars. These results indicate that morphological characteristics are not the main factor determining brittle leaf formation. Further, flower longevity among cultivars was not related to the extent of brittle leaf formation, indicating that the mechanism of reduction in flower longevity differs among cultivars. Other factors seem to be involved in variation in flower longevity caused by brittle leaf formation, such as stomatal behavior (Slootweg and van Meeteren, 1991; Mortensen and Fjeld, 1995; Mortensen and Gislerod, 1999), leaf characteristics and/or vascular structure, which was not examined in the present experiment. Further investigation is needed to clarify the genetic causes of variation in brittle leaf formation.

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Tables

Table 1. Enhancement of flower longevity in brittle-leaved ‘Asami Red’ pulsed with RNA-Ag+tris for 8 hours after simulated transport. Values shown are the average of 20 replicates \pm SD.

Leaf	Treatment	Flower longevity (days)
Normal	Control	6.5 \pm 0.1
	RNA-Ag+tris	8.6 \pm 0.6
Brittle	Control	4.5 \pm 0.2
	RNA-Ag+tris	8.9 \pm 0.5

Figures

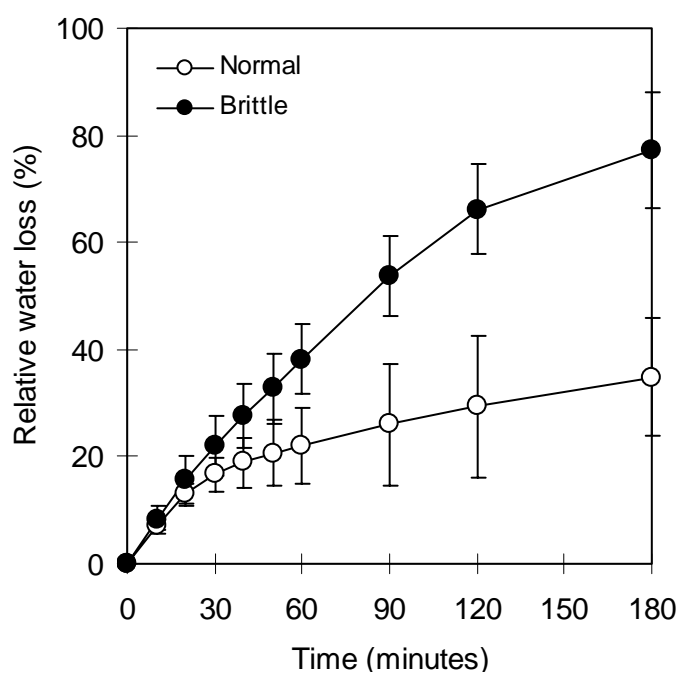


Fig. 1. Relative water loss in detached normal or brittle leaves of ‘Asami Red’. Each point is the average of 40 replicates \pm SD.

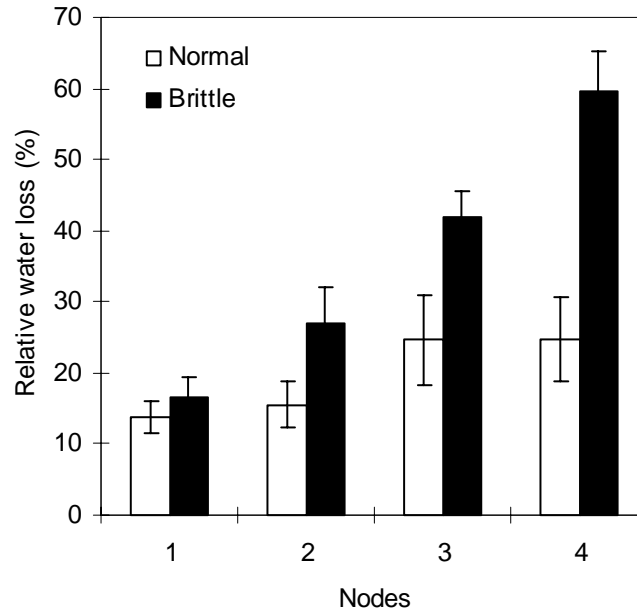


Fig. 2. Relative water loss in detached normal or brittle leaves at first to fourth nodes from apical stem. Each point is the average of 40 replicates \pm SD.

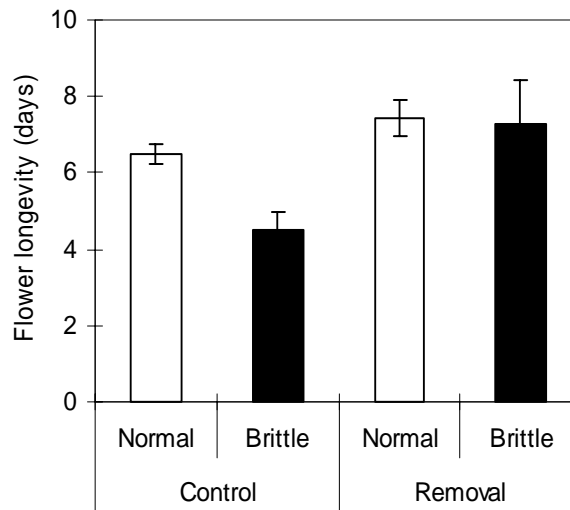


Fig. 3. Enhancement of flower longevity by removal of leaves from the third to fourth nodes. Each point is the average of 7 replicates \pm SD.

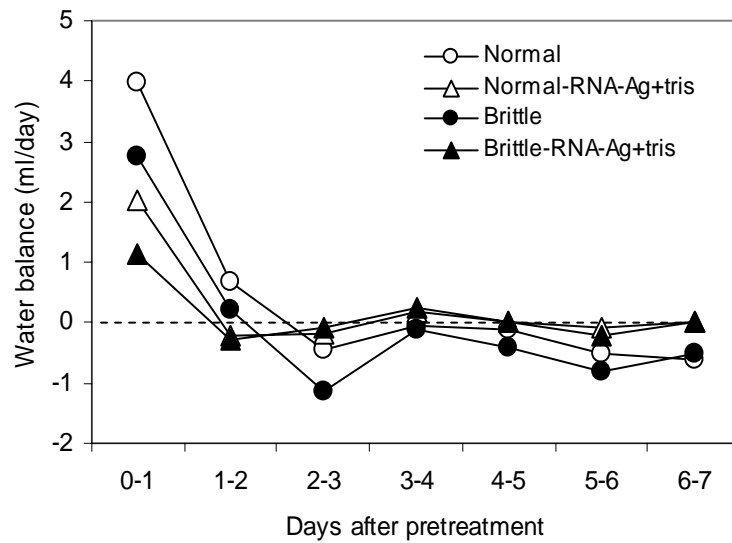


Fig. 4. Effect of RNA-Ag+tris on maintaining water balance in brittle-leaved ‘Asami Red’ cut flowers. Flower stems were pulsed with RNA-Ag+tris for 8 hours after harvest and then subjected to 18 hours of simulated transport. Water balance was calculated by subtracting FW at day n from FW at day (n+1). Values above dotted line are considered to be optimum.

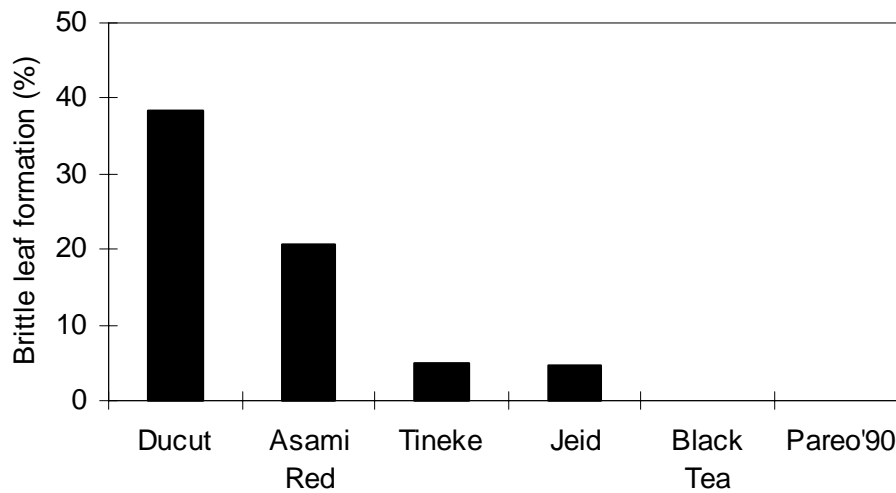


Fig. 5. Variation in brittle leaf formation among rose cultivars. Twenty flower stems were used per each cultivar.

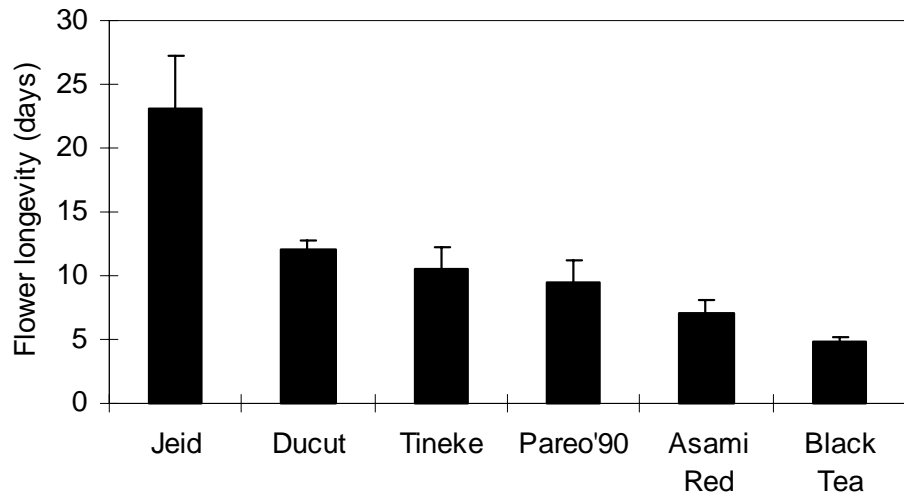


Fig. 6. Variation in flower longevity among rose cultivars grown in winter. Each point is the average of 20 replicates \pm SD.